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Favorable association between genetic polymorphisms near the *IL28B* gene and hepatic steatosis: Direct or indirect?

To the Editor,

We read with great interest the article by Tillmann *et al.* [1] that investigated the association between *IL28B* polymorphisms and hepatic steatosis, both of which are associated with a response to combination therapy with peginterferon (PEG-IFN) and ribavirin. It will provide new insight into the role of *IL28B* polymorphisms on the resistance to combination therapy against hepatitis C virus (HCV) infection.

Previous studies have reported the influence of amino acid substitutions at residue 70 of the HCV core region (from arginine to glutamine or histidine) on the resistance to combination therapy with PEG-IFN and ribavirin in patients infected with HCV genotype 1b [2–4]. In addition, prior studies have identified an association between amino acid substitutions at HCV core 70 and hepatic steatosis [5,6]. The percentage of patients with the mutant amino acid at residue 70 of the HCV core region increases with the progression of chronic hepatitis, suggesting that the mutation of the amino acid at residue 70 occurs during the natural course of chronic HCV infection [7]. Several recent studies have reported a lower prevalence of mutant amino acids at HCV core 70 in patients who have the *IL28B* polymorphism that is associated with a favorable response to combination therapy with PEG-IFN and ribavirin (i.e., CC genotype of rs12979860 and TT genotype of rs8099917) than in patients who have an unfavorable genotype [8,9]. These reports suggest that the mutation frequency of the HCV core 70 amino acid may differ according to the genetic polymorphism near the *IL28B* gene.

We analyzed polymorphisms of rs8099917 that corresponded to those of rs12979860, the rate of which is more than 99% of individuals of Japanese ethnicity [10], amino acid substitutions at HCV core 70, and hepatic steatosis based on biopsy specimens, which were obtained just prior to the start of the therapy and evaluated with the same criteria used by Tillmann *et al.*, in our 122 Japanese Mongolian patients infected with HCV genotype 1b. We found higher likelihoods of sustained virologic response in patients with the TT genotype of rs8099917, in patients with arginine at residue 70 of the HCV core region, and in patients without steatosis. We did not find significant association between *IL28B* polymorphisms and hepatic steatosis (absence of steatosis: TT genotype, 66 out of 85 (77.6%) *vs.* TG/GG genotype, 22 out of 37 (59.5%), $p = 0.0658$). We found significant associations between *IL28B* polymorphisms and the amino acid at residue 70 of the HCV core region (patients with arginine at HCV core 70: TT genotype, 71 out of 85 (83.5%) *vs.* TG/GG genotype, 14 out of 37 (37.8%), $p < 0.0001$) and also between the amino acid at HCV core 70 and hepatic steatosis (absence of steatosis: arginine at HCV core 70, 73 of 85 (85.9%) *vs.* glutamine/histidine at HCV core 70, 15 of 37 (40.5%), $p < 0.0001$). These associations may indicate that the polymorphisms near the *IL28B* gene may influence the mutation of the amino acid at residue 70 of the HCV core region, and that the amino acid mutation at HCV core 70 may influence hepatic steatosis over the course of chronic HCV infection.

There are differences between our Japanese Mongolian population and the population studied by Tillmann *et al.* including ethnicity, the rate of correspondence between rs12979860 and rs8099917, the frequency of the mutation at residue 70 of the HCV core region, and the rate of hepatic steatosis. Furthermore, the number of patients infected with HCV genotype 1b in the Tillmann *et al.* cohort is unknown. Despite these facts, it would be interesting if they were to investigate the association of amino acid substitutions at residue 70 of the HCV core both with *IL28B* polymorphisms and with hepatic steatosis.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Favorable association between genetic polymorphisms near the *IL28B* gene and hepatic steatosis: Direct or indirect?"

To the Editor:

We thank Drs. Toyoda and Kumada for raising the additional point that HCV amino acid substitutions have also been demonstrated to influence steatosis in the setting of HCV infection. In their study of 122 patients, 85 of whom had a beneficial *IL28B* genotype, Toyoda and Kumada found a trend for steatosis to be associated with *IL28B* polymorphism, as only 22% of the patients with beneficial genotype have steatosis compared to 40% of patients with the less beneficial genotype. Thus, their r, though not significant, is in line with our study, where we likewise found a 25% and 27% higher rate of steatosis in patients with the less beneficial *IL28B* ("non-C/C" for rs12979860 or "non-T/T" for rs8999017) genotype in two different cohorts of 145 and 180 patients, respectively. Similar to our and Toyoda and Kumada's results, Cai *et al.* found an association between the beneficial *IL28B* genotype and lower steatosis frequency [1]. However, a three center study by Trépo *et al.* failed to find a relevant association between *IL28B* genotype and steatosis, according to their statement [2]. This latter paper, however, did not show the data, and therefore it cannot be assessed whether the association was absent or only not significant. Toyoda and Kumada's study showed a similar trend for steatosis with *IL28B*, whereby *IL28B* is associated with different mutations in the core region, the HCV core mutation clearly shows a higher association with steatosis.

In our article, we indicate that response to treatment in relation to steatosis seems unlikely to be explained by *IL28B* alone, and though not specifically mentioned, *IL28B* is likely not solely responsible for the association with steatosis. We had a small cohort of 54 non-genotype 1 patients of whom 19 were genotype 3 and 35 were genotype 2. Despite the fact that steatosis tended to be higher in "non-C/C" patients (4/20 [20%] *vs.* 7/15 [46%] in genotype 2 patients and 4/8 [50%] *vs.* 7/11 [63%] in genotype 3 patients; Table 1) this was not significant. However, the trend was similar across genotypes. Furthermore, we have data on genotype 1a and 1b in 60 and 75 patients from the fibrosis study, respectively. In concordance with the overall results, steatosis was less frequently present in C/C genotype patients with both HCV genotype 1a and 1b (Table 1).

A possible explanation lies in the virus itself as the authors correctly point out with a focus on HCV's core protein. Unfortunately, we do not have the core antigen sequence of our patients. A previous work by Jhaveri *et al.* suggested a role for amino acids 182 and 186 of the core protein, linking steatosis *in vitro* to steatosis [3], but certainly amino acid 70 seems to be especially relevant in genotype 1b infection. There is also some evidence indicating that not all differences can be explained by viral core sequence variation [4].

Table 1. Frequency of steatosis.

		Presence of steatosis	
1A	Non-CC	19/43 (44.2%)	
	CC	2/17 (11.8%)	$p = 0.019$
1B	Non-CC	28/56 (50%)	
	CC	4/19 (21%)	$p = 0.034$
2	Non-CC	7/15 (46%)	
	CC	4/20 (20%)	n.s.
3	Non-CC	7/11 (63%)	
	CC	4/8 (50%)	n.s.

n.s., not significant.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding of conflict of interest with respect to this manuscript.

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